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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 9/10, 9/64, C12P 21/06	A1	(11) International Publication Number: WO 96/40881 (43) International Publication Date: 19 December 1996 (19.12.96)
(21) International Application Number: PCT/US96/06427 (22) International Filing Date: 8 May 1996 (08.05.96) (30) Priority Data: 08/483,151 7 June 1995 (07.06.95) US (71) Applicant: THE GENERAL HOSPITAL CORPORATION [US/US]; 55 Fruit Street, Boston, MA 02114 (US). (72) Inventors: SEED, Brian; Nine Hawthorne Place #51, Boston, MA 02114 (US). HOLGERSSON, Jan; Fatburs Kvarngata 5, S-118 64 Stockholm (SE). (74) Agent: LECH, Karen, F.; Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110-2804 (US).	(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>	
(54) Title: FUCOSYLTRANSFERASE GENES AND USES THEREOF (57) Abstract Purified DNA encoding $\alpha(1,3)$ fucosyltransferase and the recombinant proteins expressed from such DNA are disclosed. The recombinant fucosyltransferase polypeptides are used to fucosylate proteins to produce therapeutics useful for the treatment of disease, e.g., an adverse immune reaction such as septicemia or septic shock.		

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- 1 -

FUCOSYLTRANSFERASE GENES AND USES THEREOF
Statement as to Federally Sponsored Research

This invention was made at least in part with
5 funds from the Federal Government, and the Government
therefore has certain rights in the invention.

Background of the Invention

This invention relates to recombinant
fucosyltransferases, DNA, and uses thereof.

10 The structurally related endothelial cell
receptors E-selectin (Bevilacqua et al., *Proc. Natl.*
Acad. Sci. USA 84:9238, 1987; Bevilacqua et al., *Science*
243:1160, 1989) and P-selectin (Hsu-Lin et al., *J. Biol.*
Chem. 259:9121, 1984; Stenberg et al., *J. Cell Biol.*
15 101:880, 1985; Johnston et al., *Blood* 69:1401, 1987)
mediate myeloid cell attachment to the vascular wall
following activation of the endothelial cell by
inflammatory cytokines (in the case of E-selectin; see,
e.g., Bevilacqua et al. (1987), *supra*) or thrombin (in
20 the case of P-selectin; see, e.g., Geng et al., *Nature*
343:757, 1990). Expressed both by Weibel-Palade bodies
of endothelium (McEver et al., *J. Clin. Invest.* 84:920,
1989; Bonfanti et al., *Blood* 73:1109, 1989) and platelet
alpha granules (Hsu-Lin et al., *supra*; Stenberg et al.,
25 *supra*), P-selectin also mediates monocyte and neutrophil
binding to activated platelets (Larsen et al., *Cell*
59:305, 1989; Hamburger et al., *Blood* 75:550, 1990). The
leading candidate ligands for the two receptors are the
sialyl-Le^x structure for E-selectin (Lowe et al., *Cell*
30 63:475, 1990; Phillips et al., *Science* 250:1130, 1990);
Walz et al., *Science* 250:1132, 1990) and the same glycan
in the context of a specific mucin, PSGL-1, (P-selectin
glycoprotein ligand 1) for P-selectin (Sako et al., *Cell*
75:1179, 1993).

- 2 -

- Genetic and biochemical data have demonstrated the existence of at least four distinct types of fucosyltransferases capable of forming the $\alpha(1,4)$ linkage: the Lewis enzyme (Fuc-TIII), which can transfer fucose either $\alpha(1,3)$ or $\alpha(1,4)$ to Gal β 4GlcNAc or Gal β 3GlcNAc respectively (Kukowska-Latallo et al., *Genes Dev.* 4:1288, 1990) at least one enzyme, Fuc-TIV, solely forming $\alpha(1,3)$ linkages, which cannot utilize sialylated substrates (Goelz et al., *Cell* 63:1349, 1989; Lowe et al., *J. Biol. Chem.* 266:17467, 1991); at least two enzymes, Fuc-TV (Weston et al., *J. Biol. Chem.* 267:4152, 1992a) and Fuc-TVI (Weston et al., *J. Biol. Chem.* 267:24575, 1992b) solely forming $\alpha(1,3)$ linkages, which can fucosylate either sialylated or nonsialylated precursors, and a recently described enzyme, Fuc-TVII, (Sasaki et al., *J. Biol. Chem.* 269:14730, 1994); Natsuka et al., *J. Biol. Chem.* 269:16789, 1994) which can fucosylate only sialylated precursors.

Summary of the Invention

- 20 In general, the invention features substantially pure $\alpha(1,3)$ fucosyltransferase, including an amino acid sequence substantially identical to the sequence shown in Fig. 3 (SEQ ID NO: 2). In preferred embodiments, pure $\alpha(1,3)$ fucosyltransferase is obtained from a mammal (for example, a murine cell line (e.g., 32D cl3), or from a human).

- In a related aspect, the invention features a fragment or analog of $\alpha(1,3)$ fucosyltransferase polypeptide including an amino acid sequence substantially identical to the sequence shown in Fig. 3 (SEQ ID NO: 2).

In another related aspect, the invention features substantially pure DNA having a sequence substantially identical to the nucleotide sequence shown in Fig. 3 (SEQ

- 3 -

ID NO: 1). In preferred embodiments, such DNA is cDNA or is genomic DNA. In related aspects, the invention also features a vector and a cell (e.g., a murine cell such as 32D c13 or a human cell such as human cell line 293)

5 which includes such substantially pure DNA. In various preferred embodiments, the vector-containing cell is a prokaryotic cell, for example, E. coli, or, more preferably, is a eukaryotic mammalian cell (e.g., the murine cell line 32D c13 or human cell line 293).

10 In yet another related aspect, the invention features a method of fucosylating a polypeptide in vivo involving: (a) providing a cell containing the fucosyltransferase DNA of the invention including a nucleotide sequence which is substantially identical to
15 the sequence shown in Fig. 3 (SEQ ID NO: 1) positioned for expression in the cell; and (b) culturing the transformed cell under conditions for expressing the DNA, resulting in the fucosylation of the protein.

In preferred embodiments, fucosylation occurs in a
20 mammalian cell, for example, a human cell (e.g., human cell line 293) or a murine cell (e.g., 32D c13). In related aspects, the cell contains a second fucosyltransferase gene. Preferably, such a second gene is substantially identical to the nucleotide sequence
25 shown in Fig. 6A (SEQ ID NO: 3) which encodes a polypeptide including an amino acid sequence substantially identical to the sequence shown in Fig. 6B (SEQ ID NO: 4). In preferred embodiments, the protein which is fucosylated according to the above method is an
30 AGP-antibody fusion protein or is an antibody (e.g., IgG or IgM).

In another aspect, the invention features a recombinant polypeptide fucosylated using a cell expressing DNA which is substantially identical to the
35 nucleotide sequence shown in Fig. 3 (SEQ ID NO: 1).

- 4 -

Preferably, the fucosylated polypeptide is an AGP-antibody fusion protein, or is an antibody (e.g., IgG or IgM). In still other preferred embodiments, the polypeptide is further fucosylated using a second
5 fucosyltransferase. Such a second fucosyltransferase is substantially identical to a polypeptide including an amino acid sequence shown in Fig. 6B (SEQ ID NO: 4). Preferably, the cell used to fucosylate the polypeptide is a mammalian cell (e.g., the murine cell line 32D c13
10 or the human cell line 293).

In another aspect, the invention features a polypeptide fucosylated in vitro using a fucosyltransferase having an amino acid sequence substantially identical to the sequence shown in Fig. 3
15 (SEQ ID NO: 2). In preferred embodiments, the fucosylated polypeptide is further fucosylated using a second fucosyltransferase. Such a second fucosyltransferase includes an amino acid sequence substantially identical to the sequence shown in Fig. 6B
20 (SEQ ID NO: 4). Preferably, the fucosylated polypeptide is an AGP-antibody fusion protein or is an antibody (e.g., IgG or IgM).

In related aspects, the invention features a substantially pure polypeptide of the invention which is
25 fucosylated in vivo or in vitro and which is capable of protecting a mammal against an adverse immune reaction. In general, such an adverse immune reaction is septic shock or is septicemia.

In another aspect, the invention features a cell
30 containing at least two recombinant fucosyltransferases, one of the fucosyltransferases being substantially identical to the amino acid sequences shown in Fig. 3. (SEQ ID NO: 2) and another of the fucosyltransferases being substantially identical to the amino acid sequence

- 5 -

shown in Fig. 6B (SEQ ID NO: 4). Preferably, such a DNA-containing cell is a

prokaryotic cell (e.g., E. coli) or is a eukaryotic cell, for example, a mammalian cell (e.g., the murine cell line

5 32D c13 or human cell line 293).

In a final aspect, the invention features a method of fucosylating a polypeptide in vitro comprising: (a) providing an $\alpha(1,3)$ fucosyltransferase of the invention; and (b) contacting the polypeptide with the

10 fucosyltransferase under conditions sufficient for fucosylating the polypeptide.

By "polypeptide" is meant any chain of amino acids, regardless of length or post-translational modification (e.g., glycosylation or phosphorylation).

15 By "substantially identical" is meant a polypeptide exhibiting at least 50%, preferably 70%, more preferably 90%, and most preferably 95% homology to a reference amino acid or is meant a nucleic acid sequence exhibiting at least 85%, preferably 90%, more preferably
20 95%, and most preferably 97% homology to a reference nucleic acid sequence. For polypeptides, the length of comparison sequences will generally be at least 16 amino acids, preferably at least 20 amino acids, more preferably at least 25 amino acids, and most preferably
25 35 amino acids. For nucleic acids, the length of comparison sequences will generally be at least 30 nucleotides, preferably at least 60 nucleotides, more preferably at least 75 nucleotides, and most preferably 110 nucleotides.

30 Sequence identity is typically measured using sequence analysis software (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). Such software
35 matches similar sequences by assigning degrees of

- 6 -

homology to various substitutions, deletions, substitutions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, 5 leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

By a "substantially pure polypeptide" is meant a fucosyltransferase polypeptide which has been separated 10 from components which naturally accompany it. Typically, the polypeptide is substantially pure when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, 15 more preferably at least 90%, and most preferably at least 99%, by weight, fucosyltransferase polypeptide. A substantially pure fucosyltransferase polypeptide may be obtained, for example, by extraction from a natural source (e.g., a murine cell such as 32D c13); by 20 expression of a recombinant nucleic acid encoding a fucosyltransferase polypeptide; or by chemically synthesizing the protein. Purity can be measured by any appropriate method, e.g., column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

25 A protein is substantially free of naturally associated components when it is separated from those contaminants which accompany it in its natural state. Thus, a protein which is chemically synthesized or produced in a cellular system different from the cell 30 from which it naturally originates will be substantially free from its naturally associated components. Accordingly, substantially pure polypeptides include, without limitation, those derived from eukaryotic organisms but synthesized in E. coli or other 35 prokaryotes, or those derived from a eukaryotic cell

- 7 -

which does not normally synthesize such a protein, or those derived from a eukaryotic cell engineered to overexpress such a protein.

By "substantially pure DNA" is meant DNA that is
5 free of the genes which, in the naturally-occurring genome of the organism from which the DNA of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously
10 replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or which exists as a separate molecule (e.g., a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. It also includes a
15 recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

By "transformed cell" is meant a cell into which (or into an ancestor of which) has been introduced, by means of recombinant DNA techniques, a DNA molecule
20 encoding (as used herein) a fucosyltransferase polypeptide.

By "positioned for expression" is meant that the DNA molecule is positioned adjacent to a DNA sequence which directs transcription and translation of the
25 sequence (i.e., facilitates the production of, e.g., a recombinant fucosyltransferase polypeptide or RNA molecule).

By "promoter" is meant the minimal sequence sufficient to direct transcription. Also included in the
30 invention are those promoter elements which are sufficient to render transcription controllable for cell-type specific, tissue-specific, or inducible expression; such elements may be located in the 5' or 3' regions of the native gene.

- 8 -

By "operably linked" is meant that a gene and a regulatory sequence(s) are connected in such a way as to permit gene expression when the appropriate molecules (e.g., transcriptional activator proteins) are bound to the regulatory sequence(s).

By "purified antibody" is meant antibody which is at least 60%, by weight, free from proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably 90%, and most preferably at least 99%, by weight, antibody, e.g., a fucosyltransferase-specific antibody. A purified fucosyltransferase antibody may be obtained, for example, by affinity chromatography using recombinantly-produced fucosyltransferase protein or conserved motif peptides and standard techniques.

By "specifically binds" is meant an antibody which recognizes and binds a fucosyltransferase protein but which does not substantially recognize and bind other molecules in a sample, e.g., a biological sample, which naturally includes fucosyltransferase.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

25

Detailed Description

The drawings will first be described.

Drawings

Figure 1 is a flow cytometry profile showing the expression of fucosylated glycans by COS cells transfected with murine myeloid fucosyltransferase. The results are expressed as mean fluorescence intensity in arbitrary units. No expression above background of any carbohydrate epitope except sialyl-Le^x was seen.

- 9 -

Figures 2A-C are panels of autoradiograms showing different nucleic acid blot hybridizations. Panel (A) is an autoradiogram showing a DNA blot hybridization of total genomic DNA from mouse kidney tissue. Murine genomic DNA (15 μ g) was digested with the indicated restriction enzymes and subjected to fractionation, transfer, and blot hybridization. Panel (B) is an autoradiogram showing an RNA blot hybridization of total RNA from different cell lines. Total RNA (20 μ g) prepared from each of the cell lines shown was denatured, fractionated by gel electrophoresis, transferred to nylon and hybridized. The lineage origins of the cell lines are: YAC-1 (T cell leukemia), EL4 (thymoma), RDM4 (T cell leukemia), CTLL-2 (IL-2 dependent cytotoxic T cell), 32D cl3 (IL-3 dependent granulocyte precursor), WEHI-231 (B cell lymphoma, non-secreting, mouse), WEHI-279 (B cell lymphoma, non-secreting, mouse), Sp2/0 (plasmacytoma), WEHI-3B (myeloid (monocytic) leukemia), P815 (mastocytoma), Ltk (fibroblast), and Balb 3T3 (fibroblast). Panel (C) is an autoradiogram showing an RNA blot hybridization of total RNA from skeletal tissue. Total RNA (20 μ g) prepared from skeletal muscle (Sk. muscle) was denatured, fractionated by gel electrophoresis, transferred to nylon and hybridized. Arrows denote the location of ribosomal RNAs.

Figure 3 is the nucleotide (SEQ ID NO: 1) and deduced amino acid sequence (SEQ ID NO: 2) of the murine myeloid-lineage fucosyltransferase cDNA. Two sites for N-linked glycan addition are underlined in the predicted peptide sequence, as is the upstream ATG in the nucleic acid sequence.

Figure 4 is a flow cytometry profile showing the expression of fucosylated glycans by 32D cl3 cells stably transfected with the human myeloid fucosyltransferase. Pooled products of the transfection of 32D cl3 cells with

- 10 -

a human Fuc-TIV myeloid fucosyltransferase expression plasmid bearing a selectable marker were evaluated by indirect immunofluorescence using anti-carbohydrate monoclonal antibodies and flow cytometry. The results are expressed as mean fluorescence intensity in arbitrary units.

Figure 5 is a bar graph of cell adhesion assays (Panels A-B). Panel (A) shows the adhesion to E-selectin or P-selectin IgG fusion proteins of COS cells transfected with either the human Fuc-TIV (FTIV) or the murine 32D cl3 fucosyltransferase (32DFT) cDNAs in the presence or absence of the P-selectin glycoprotein ligand (PSGL). Panel (B) shows adhesion to E-selectin or P-selectin IgM fusion proteins of 32D cl3 cells (32D cl3) or 32D cl3 cells transfected with the human myeloid Fuc-TIV cDNA (FTIV). TLISA, control IgM fusion protein; E, P, E- or P-selectin IgM fusion protein. Binding to P-selectin/IgM fusion protein was not significantly increased by expression of Fuc-TIV. Columns represent average cpm bound of triplicate samples.

Figure 6, panel A is the nucleotide sequence (SEQ ID NO: 3) and, panel B, the deduced amino acid sequence (SEQ ID NO: 4) of the human myeloid-lineage Fuc-TIV cDNA.

Below we describe the isolation of an $\alpha(1,3)$ fucosyltransferase cDNA from a murine myeloid cell line which has a relatively strict substrate requirement for sialylated N-acetylactosamine, which can account for the presence of the sialyl-Le^x epitope on murine cells, and which is substantially more effective than the previously described myeloid cell fucosyltransferase, Fuc-TIV, in support of E-selectin-mediated COS cell adhesion. We also show that the introduction of the human Fuc-TIV transferase into a murine cell line results in the appearance of a fucosylated glycan pattern similar to that found on human neutrophils and monocytes. Murine

- 11 -

cells expressing human Fuc-TIV show enhanced adhesion to E-selectin fusion proteins, indicating that Fuc-TIV has an important physiological function in the context of human granulocyte extravasation. These examples are presented to illustrate, not limit, the invention.

Cell Lines and Growth Conditions

The following cell lines and growth conditions were used in this study. YAC-1, EL4, RDM4, CTLL2, Sp2/0, WEHI-231, WEHI-279 and P815 cell lines were cultured in 10% IMDM, 10% FCS, 50 mM mercaptoethanol, 50 U/ml penicillin and 50 µg/ml streptomycin. Balb 3T3, Ltk⁻ and COS-7 m6 cell lines were passaged in DMEM, 10% calf serum (CS) and 25 µg/ml gentamicin sulfate. The IL-3 producing, macrophage-like cell line WEHI-3B was cultured in RPMI-1640, 10% FCS, 50 U/ml penicillin and 50 mg/ml streptomycin. The IL-3 dependent mouse neutrophil progenitor cell line, 32D c13, was cultured in RPMI-1640, 10% FCS, 10% WEHI 3B-conditioned medium, 50 U/ml penicillin and 50 µg/ml streptomycin (Kreider et al., *Oncogene* 7:135, 1992).

Isolation of cDNA Encoding Murine α(1,3) Fucosyltransferase

To isolate a cDNA clone capable of directing the expression of sialyl-Le^x determinants, an expression library was prepared from mRNA isolated from the murine cell line 32D c13, which phenotypically resembles a granulocyte precursor and which binds murine E- and P-selectin (Levinovitz et al., *J. Cell Biol.* 121:449, 1993) as follows. A cDNA library in the expression vector CDM8 was prepared from 32D c13 cells as described by Aruffo et al. (*Proc. Natl. Acad. Sci. USA* 84:8573, 1987) using the introduction of a Mung bean exonuclease treatment followed by T4 DNA polymerase prior to the addition BstX1 adaptors. Following the second strand synthesis, the

- 12 -

cDNA pellet was resuspended in 225 μ l distilled water, 25 μ l 10X Mung bean incubation buffer (500 mM sodium acetate, 300 mM NaCl, 10 mM zinc sulfate, pH 5.0), and 10 U of Mung bean nuclease (New England Biolabs, Beverly, MA.). After a 10 minute incubation period at 37°C, the reaction was stopped by adding 20 μ l of 1 M Tris-HCl, pH 8.0 and 3 μ l 0.5 M EDTA, pH 8.0. The cDNA was phenol extracted, ethanol precipitated and resuspended in 90 μ l distilled H₂O. Following the addition of 10 μ l 10x T4 DNA polymerase buffer (330 mM Tris acetate, 660 mM potassium acetate, 100 mM magnesium acetate, 5 mM DTT, 1 mg/ml bovine serum albumin (BSA), pH 8.0), 1 μ l of a mixture containing 25 mM each of the deoxynucleoside triphosphates and 1 U of T4 DNA polymerase (Boehringer-Mannheim), the mixture was incubated at 37°C for 30 min. The reaction was stopped by adding 0.5 μ l 0.5 M EDTA, phenol extracted and ethanol precipitated. Following ligation of the BstX1 adaptors, unligated adaptors were removed by repeated centrifugal ultrafiltration through a filter with a molecular weight cut off of 100 kD (Amicon, Danvers, MA), followed by velocity sedimentation fractionation.

The ligated cDNA in CDM8 was introduced into electrocompetent MC1061/p3 cells by electroporation in 0.2 cm gap cuvettes (Bio-Rad laboratories, Hercules, CA) at a voltage of 2.5 kV, a capacitance of 25 μ F and a parallel resistance of 400 Ohms. Transformed bacteria were plated on 20 dishes, 23 x 23 cm in size (Nunc, Denmark). Bacteria from each dish ($\approx 1.25 \times 10^5$ colonies) were harvested and an aliquot stored frozen at -70°C in 40% glycerol. Plasmid DNA was isolated from each pool using a commercial kit (plasmid midi prep QIAGEN Inc., Chatsworth, CA) according to the manufacturer's recommendations.

- 13 -

The library was then divided into 20 pools of 1.25×10^5 cells each and between 200 and 500 ng of plasmid DNA from each of the 20 pools was separately transfected into COS-7 m6 cells at approximately 70% confluence in a 10 cm-dish using the DEAE-dextran method described by Seed et al. (*Proc. Natl. Acad. Sci USA* 84:3365, 1987). The COS cells were stained with the sialyl-Le^x antibody and bacteria from positive pools replated at lower density. Between 48 and 60 hours post-transfection pools bearing cDNAs capable of directing the appearance of the sialyl-Le^x determinant, dishes with positive cells were identified by immunocytochemistry using an anti-sialyl-Le^x antibody (KM93, mouse IgM; Kamiya Biomedical Company, Thousand Oaks, CA), and an avidin-biotin complex protocol employing 9-amino-3-ethylcarbazol as a peroxidase substrate kit (Vector Labs, Burlingame, CA) essentially as described Horst et al. (*Nucleic Acids Res.* 19:4556, 1991; Vector Labs). Bacteria corresponding to positive pools were subsequently replated at lower density on 10 cm dishes. Plasmid DNA from these subpools was transfected into COS cells in 6 cm dishes. The procedure was repeated until a single plasmid was recovered that conferred binding of anti-sialyl-Le^x antibody to transfected COS cells. Bacterial cells from the pool giving rise to the highest number of positive transfectants were plated at lower density on agar plates and DNA prepared from the bacteria was transfected into COS cells, allowing pools of successively less sequence complexity to be obtained until finally a single clonally pure plasmid isolate was shown to be capable of directing the appearance of the sialyl Lewis-X epitope in COS cells. Five out of twenty pools contained five or more positive cells.

- 14 -

Flow Cytometry Analysis

To confirm the expression of the sialyl Lewis-X epitope, we examined the expression of this epitope in COS cells transfected with the 32D c13 fucosyltransferase expression plasmid described above by indirect immunofluorescence using anti-carbohydrate monoclonal antibodies and flow cytometry as follows. Transfected COS cells to be stained for FACS analysis were harvested by detaching the cells from plates 48 to 60 hours post-transfection using 0.5 mM EDTA in phosphate-buffered saline (PBS). Staining of cells for FACS analysis was done by incubating 2×10^6 cells on ice for 20 to 30 minutes in 0.5 ml of 3% BSA in PBS with 4 μ g/ml of antibody or in 0.5 ml of hybridoma supernatant for 20 to 30 minutes. For this analysis, the following antibodies were used: anti-Le^x antibody (PM81, mouse IgM; Medarex, Inc., West Lebanon, NH), anti-sialyl-Le^x (KM93, mouse IgM), anti-sialyl-Le^a (KM231, mouse IgG1; Kamiya Biomedical Co., Thousand Oaks, CA), anti-Le^a antibody (T174, mouse IgG1; Signet, Dedham, MA), anti-CD65 (VIM-2) antibody (88H7, mouse IgM; AMAC, Westbrook, ME), and the hybridoma secreting a mouse IgG3 antibody against di- and tri- fucosylated Le^x (FHCR-1-2075/FH4; ATCC, Rockland, MD). Following two washes in PBS, the cells were resuspended in 0.5 ml of 3% BSA in PBS containing 2 μ g/ml FITC-conjugated anti-mouse IgG or IgM antibody (Organon Teknika Corp., Durham, NC). Washed cells were immediately analyzed by flow cytometry (Coulter Corp., Hialeah, FL) according to standard methods. Flow cytometric analysis of the transfected cells showed that sialyl-Le^x, but neither Le^x, CD65, di/trimeric Le^x (FH4 epitope), Le^a, nor sialyl-Le^a determinants could be detected (Fig. 1).

- 15 -

DNA Blot (Southern) Hybridization Analysis

- To determine gene copy number of the 32D c13 fucosyltransferase gene, DNA blot hybridization was performed as follows. Fifteen micrograms of mouse genomic DNA (Adult, male, Balb/c kidney; Clontech Labs, Palo Alto, CA) was digested overnight in a volume of 300 μ l with 50 U of BamHI, 90 U of EcoRI, 100 U of HindIII and 100 U of PstI individually. The digests were phenol extracted, ethanol precipitated and separated on a 0.8% agarose gel. The gel was denatured by incubation in 0.5 M NaOH, 1.5 M NaCl, at room temperature for 30 minutes, briefly rinsed in distilled water, and neutralized for 30 minutes in 0.5 M Tris-HCl, pH 7.0, 1.5 M NaCl at room temperature. Following an incubation in 20X SSC for 30 minutes, the DNA was blotted and probed as for the RNA blots described below. The results of this analysis indicated that the fucosyltransferase is encoded by at least a single copy gene (Fig. 2A).

RNA Blot Hybridization Analysis

- To study the expression of the 32D c13 fucosyltransferase gene, RNA blot hybridization was performed as follows. Total RNA was isolated from YAC-1, EL4, RD-M4, CTLL2, 32D c13, WEHI 231, WEHI 279, Sp2/0, WEHI 3B, P815, Ltk- and Balb 3T3 cells using a guanidinium-acid phenol protocol (Chomczynski et al., *Analyt. Biochem.* 162:156, 1987). The heart, stomach, small intestine, large intestine, liver, kidneys, spleen, brain and a piece of skeletal muscle was dissected from a male 129 SVJ mouse sacrificed by cervical dislocation.
- Total RNA was isolated from the tissues as described above after homogenization on ice in guanidinium thiocyanate buffer using a handheld homogenizer (Omni International, Waterbury, CT). Twenty micrograms of total RNA was separated by electrophoresis in a 1.2%

- 16 -

- agarose/formaldehyde gel and transferred to nylon membranes (Schleicher & Schuell, Keene, N.H.) using a downward transfer system (Schleicher & Schuell) according to the manufacturer's recommendations. RNA absorbed to
- 5 the membrane was crosslinked by UV irradiation (1200 μ J) and detected by hybridization with a randomly primed probe using standard conditions (Ausubel et al., *Current Protocols In Molecular Biology*, Wiley Interscience, 1995).
- 10 RNA blot analysis showed a pattern of highly tissue-restricted expression of a message of 1.9 kb (Fig. 2 B and C). Among established cell lines high levels of mRNA were found in 32D cl3 and CTLL-2, an IL-2 dependent cytotoxic T cell line, with the myeloid cell line WEHI 3B
- 15 and the mastocytoma P815 having significant quantities of the mRNA (Fig. 2B). No message was detected in T-cell lines (YAC-1, EL4 and RDM4), in B cell or fibroblast lines, or in any of the tissues sampled (Fig. 2B).

DNA Sequencing

- 20 The sequence of the isolated cDNA clone was determined by the dideoxy chain termination method using modified T7 DNA polymerase (U.S. Biochemical Corp., Cleveland, OH) (Sanger et al., *Proc. Natl. Acad. Sci. USA* 74:5463, 1977). Ambiguities found in the sequence due to
- 25 self-complementary sequences were resolved using 7-deaza-dGTP.

DNA Sequence Analysis and Deduced Protein Sequence

- The cDNA insert consists of 1814 nucleotides, terminating just 3' to a canonical upstream poly(A)
- 30 sequence motif (Fig. 3). The largest open reading frame begins at a methionine at position 325 which does not meet the sequence requirements for a translational initiation consensus, but is preceded by only one other

- 17 -

- candidate ATG, similarly lacking a consensus initiation context and giving rise to a translation product which terminates within a few residues. The predicted polypeptide contains a long amino-terminal hydrophobic region preceded by arginine residues, similar in structure to the transmembrane domain of type II (amino terminally anchored) integral membrane proteins typical of this class of glycosyltransferase. A carboxyl-terminal sequence from peptide residues 245 to 258
- 10 consisting largely of aliphatic hydrophobic amino acids, although reminiscent of the membrane spanning domains of type I integral membrane proteins, is probably too short to serve a membrane insertion function. The predicted molecular mass of the encoded protein is 39.4
- 15 kilodaltons, with the presence of two N-linked glycan addition sites at residues 81 and 291 suggesting that the mature protein may be larger.

Comparison of protein sequences showed that the 32D c13 fucosyltransferase shares identity at 47% of

20 residues with the human myeloid fucosyltransferase and at 80% of residues with the human type VII fucosyltransferase. Given that the N-acetyllactosamine fucosyltransferases constitute a large and growing family, it is likely that another unidentified human

25 isolate may prove more closely related to the present 32D c13 fucosyltransferase enzyme. Such a gene is isolated using the techniques described herein.

Expression of Human Fucosyltransferase in Murine Cells

- The cDNA insert of a previously isolated
- 30 expression clone encoding human myeloid $\alpha(1,3)$ fucosyltransferase (Fuc-TIV; Fig. 6A, SEQ ID NO: 3) was excised from the Ψ H3m vector (Aruffo et al. *Proc. Natl. Acad. Sci. USA* **84**:8573, 1987) with HindIII and HpaI and subcloned into the polylinker of a bidirectional vector

- 18 -

bearing the Spleen-focus forming virus (Sffv) LTR upstream of a polylinker, a splice donor and acceptor site, and the bidirectional poly(A) addition signal from SV40; opposite in orientation to this transcription unit, and utilizing the poly(A) signals from the opposite direction was a second transcription unit consisting of the HSV TK promoter followed by the coding sequences for puromycin acetyltransferase. The Sffv Fuc-TIV plasmid was linearized by digestion with Avr2, phenol extracted, ethanol precipitated, and electroporated into the 32D cl3 cell line as follows. The cells (8×10^7) were resuspended in 0.8 ml RPMI-1640, 10% FBS, 10% WEHI-3B conditioned medium, and transferred together with 40 μ g of linearized plasmid DNA to a 0.4 cm-gap electroporation cuvette (Bio-Rad, Hercules, CA) on ice. A single pulse was delivered at a voltage of 250V and a capacitance of 500 μ F. After electroporation, the cuvette was put back on ice for 10 minutes before the cells were transferred to a flask containing 50 ml of medium. Puromycin was added to the medium the following day at a concentration of 0.5 μ g/ml. After approximately 2 weeks, with media changes every second to third day, the cells were checked for expression of the sialyl-Le^x epitope.

Transfection of the human myeloid cell-specific fucosyltransferase cDNA (Fuc-TIV; Fig. 6A, SEQ ID NO: 3) into a murine granulocytic cell line resulted in the appearance of glycan epitope pattern similar to that of human cells (Fig. 4). Specifically, the levels of expression of Le^x (CD15) and NeuNAc α 3Gal β 4GlcNAc β 3Gal β 4(Fu α 3)GlcNAc (CD65) epitopes were markedly increased, and substantial levels of multiply fucosylated poly N-acetyllactosamine epitopes were also detected. Together these findings support the notion that internally fucosylated poly N-

- 19 -

acetyllactosamine chains are present in the transfectant but not parental cells.

Fusion Proteins

- The construction of DNA sequences coding for fusions between E- and P-selectin extracellular domains (for P-selectin only 2 of the complement regulatory domains were included) and the Fc part (hinge, CH2 and CH3) of human genomic IgG1 was performed as previously described (Walz et al., *Science* **250**:1132, 1990; Aruffo et al., *Cell* **61**:1303, 1990). The cDNA sequences for E- and P-selectin extracellular domains were fused to the genomic sequence of human IgM Fc (CH2, CH3 and CH4) by transferring the selectin sequences from an IgG fusion vector to an IgM fusion vector created in this laboratory (Zettlmeissl et al., *DNA Cell Biol.* **9**:347, 1990). The PSGL-1 cDNA coding sequence was obtained by PCR from an HL-60 cDNA library and confirmed by DNA sequencing. The coding segment for the mature extracellular, transmembrane and intracellular domain was inserted in an expression vector based on CDM8 which lacks the polyoma virus origin of replication and contains the leader sequence for the CD5 antigen positioned just upstream of the coding region for an influenza hemagglutinin peptide epitope tag.
- COS cell supernatants containing soluble E- and P-selectin/IgG and IgM fusion proteins were produced as previously described (Walz et al., *Science* **250**:1132, 1990; Aruffo et al., *Cell* **61**:1303, 1990). The concentration of fusion protein in the tissue culture supernatants was determined by a 96-well ELISA assay, in which the fusion proteins were captured with an affinity purified, polyclonal anti-human IgG Fc or anti-human IgM (μ chain specific) antibody (Organon Teknika, Durham, NC). Captured fusion proteins were detected with a

- 20 -

peroxidase-conjugated, affinity purified, polyclonal anti-human IgG Fc or anti-human IgM (μ chain specific) antibody (Organon Teknika) using O-phenylenediamine dihydrochloride as substrate (Sigma). The ELISA was calibrated using purified human IgG or IgM (Sigma).

Adhesion Assays

- Adhesion assays were performed in 96-well ELISA plates (Becton-Dickinson, Oxnard, CA) as follows. The wells were incubated with 100 μ l of 20 μ g/ml anti-human
- 10 IgG Fc or anti-human IgM (heavy chain specific) in PBS for 2 hrs in a humid chamber at room temperature. After washing the plate twice with PBS, additional protein-binding sites were blocked by an overnight incubation with 200 μ l 3% BSA in PBS. The plate was washed with PBS
 - 15 four times and incubated with 200 μ l of fusion protein supernatants for 2 hrs. Following three PBS washes and one wash in 0.2% BSA, 0.15 M NaCl, 3 mM CaCl₂, 2×10^5 cells/well in 200 μ l 0.2% BSA, 0.15 M NaCl, 3 mM CaCl₂ were added and left to bind for 15 minutes in room
 - 20 temperature having the plate on a rotary platform (60 rpm). The plate was washed three times by carefully dropping in 200 μ l 0.15 M NaCl, 3 mM CaCl₂ in the wells and then carefully inverting the plate on a pile of paper towels in order to gently pour out the liquid.
 - 25 Transfected cells used for the assay were lifted off the dish with 0.5 mM EDTA in PBS 48 to 60 hrs after transfection and loaded with 100 μ l Na₂⁵¹CrO₄ (1 mCi/ml; DuPont, Boston, MA) in 0.9% NaCl plus 100 ml medium at 37°C for 1 hr. Loaded cells were washed twice in PBS and
 - 30 resuspended in 0.2% BSA, 0.15 M NaCl, 3 mM CaCl₂. Adherent cells were lysed by the addition of 200 μ l 2% SDS and counted in a gamma ray spectrometer.

The results of these experiments showed that COS cells transfected with the 32D c13 fucosyltransferase

- 21 -

CDNA had significantly greater binding to immobilized E- and P-selectin than did the human Fuc-TIV (myeloid) enzyme; the binding to P-selectin was observed only when the COS cells were cotransfected with a cDNA expression
5 vector encoding PSGL-1 (Fig. 5A).

The 32D cl3 cell line itself binds both human E- and P-selectin/IgM fusion proteins (Fig. 5B). IgM fusion proteins were used in these experiments to avoid the possible contribution of Fc receptor binding. To
10 evaluate the functional consequences of a human-like fucosylated glycan spectrum, 32D cl3 cells stably expressing the human Fuc-TIV (myeloid) enzyme (Fig. 6B) were evaluated in the selectin adhesion assay. The transfectants showed an approximately 10-fold higher
15 binding density to human E-selectin relative to untransfected cells, whereas binding to P-selectin was not significantly affected (Fig. 5).

Isolation of Fucosyltransferase cDNA and Genomic DNA

Cloning and isolation of fucosyltransferase cDNA
20 according to the invention is carried out according to the methods described herein. Cloning of genomic DNA is performed according to well known methods.

Based on our discovery of a novel myeloid-expressed fucosyltransferase, the isolation of additional
25 mammalian fucosyltransferases, including human fucosyltransferases, is made possible using standard techniques. In particular, using all or a portion of the amino acid sequence of a fucosyltransferase of the invention, one may readily design fucosyltransferase
30 oligonucleotide probes, including fucosyltransferase degenerate oligonucleotide probes (i.e., a mixture of all possible coding sequences for a given amino acid sequence). These oligonucleotides may be based upon the sequence of either strand of the DNA comprising the

- 22 -

motif. General methods for designing and preparing such probes are provided, for example, in Ausubel et al., supra, and Guide to Molecular Cloning Techniques, 1987, S. L. Berger and A. R. Kimmel, eds., Academic Press, New York. These oligonucleotides are useful for fucosyltransferase gene isolation, either through their use as probes capable of hybridizing to fucosyltransferase complementary sequences or as primers for various polymerase chain reaction (PCR) cloning strategies. In one particular example, isolation of other fucosyltransferase genes is performed by PCR amplification techniques well known to those skilled in the art of molecular biology using oligonucleotide primers designed to amplify only sequences flanked by the oligonucleotides in genes having sequence identity to fucosyltransferase of the invention. The primers are optionally designed to allow cloning of the amplified product into a suitable vector.

Hybridization techniques and procedures are well known to those skilled in the art and are described, for example, in Ausubel et al., supra, and Guide to Molecular Cloning Techniques, supra. If desired, a combination of different oligonucleotide probes may be used for the screening of the recombinant DNA library. The oligonucleotides are labelled with ^{32}P using methods known in the art, and the detectably-labelled oligonucleotides are used to probe filter replicas from a recombinant DNA library. Recombinant DNA libraries may be prepared according to methods well known in the art, for example, as described in Ausubel et al., supra, or may be obtained from commercial sources.

For detection or isolation of closely related fucosyltransferases, high stringency conditions may be used; such conditions include hybridization at about 42°C and about 50% formamide; a first wash at about 65°C,

- 23 -

- about 2X SSC, and 1% SDS; followed by a second wash at about 65°C and about 0.1% SDS, 1X SSC. Lower stringency conditions for detecting fucosyltransferase genes having about 85% sequence identity to the fucosyltransferase
- 5 gene described herein include, for example, hybridization at about 42°C in the absence of formamide; a first wash at about 42°C, about 6X SSC, and about 1% SDS; and a second wash at about 50°C, about 6X SSC, and about 1% SDS.
- 10 As discussed above, fucosyltransferase oligonucleotides may also be used as primers in PCR cloning strategies. Such PCR methods are well known in the art and described, for example, in *PCR Technology*, H.A. Erlich, ed., Stockton Press, London, 1989; *PCR*
- 15 *Protocols: A Guide to Methods and Applications*, M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White, eds., Academic Press, Inc., New York, 1990; and Ausubel et al., supra. If desired, fucosyltransferases may be isolated using the PCR "RACE" technique, or Rapid Amplification of
- 20 cDNA Ends (see, e.g., Innis et al., supra). By this method, oligonucleotide primers based on a fucosyltransferase conserved-domain are oriented in the 3' and 5' directions and are used to generate overlapping PCR fragments. These overlapping 3'- and 5'-end RACE
- 25 products are combined to produce an intact full-length cDNA. This method is described in Innis et al., supra; and Frohman et al., *Proc. Natl. Acad. Sci. USA* 85:8998, 1988.

Fucosyltransferase Polypeptide Expression

- 30 Fucosyltransferases according to the invention may be expressed or produced by transformation of a suitable host cell with all or part of a fucosyltransferase-encoding cDNA fragment (e.g., the cDNA described herein)

- 24 -

in a suitable expression vehicle (e.g., those described herein).

- Those skilled in the field of molecular biology will understand that any of a wide variety of expression systems may be used to provide the recombinant protein. For example, a fucosyltransferase may be produced in a prokaryotic host (e.g., E. coli) or in a eukaryotic host (e.g., Saccharomyces cerevisiae or mammalian cells, e.g., 32D c13, human cell line 293, COS 1, NIH 3T3, and JEG3 cells). Such cells are available from a wide range of sources (e.g., the American Type Culture Collection, Rockland, MD; also, see, e.g., Ausubel et al., supra). The method of transformation and the choice of expression vehicle will depend on the host system selected.
- 15 Transformation methods are described, e.g., in Ausubel et al. (supra); expression vehicles may be chosen from those provided, e.g., in Cloning Vectors: A Laboratory Manual (P.H. Pouwels et al., 1985, Supp. 1987).

- One preferred expression system is the mouse 3T3 fibroblast host cell transfected with a pMAMneo expression vector (Clontech, Palo Alto, CA). pMAMneo provides: an RSV-LTR enhancer linked to a dexamethasone-inducible MMTV-LTR promoter, an SV40 origin of replication which allows replication in mammalian systems, a selectable neomycin gene, and SV40 splicing and polyadenylation sites. DNA encoding a fucosyltransferase polypeptide is inserted into the pMAMneo vector in an orientation designed to allow expression. The recombinant fucosyltransferase is isolated as described below. Other preferable host cells which may be used in conjunction with the pMAMneo expression vehicle include COS cells and CHO cells (ATCC Accession Nos. CRL 1650 and CCL 61, respectively).
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- 25
- 30

- More preferably, fucosyltransferase of the invention is expressed or produced by a stably-
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- 25 -

transfected mammalian cell line (e.g., 32D c13, or human cell line 293) using the methods and vectors described herein.

In addition, a number of other vectors suitable for stable transfection of mammalian cells are available to the public, e.g., see Pouwels et al. (supra); methods for constructing such cell lines are also publicly available, e.g., in Ausubel et al. (supra). In one example, cDNA encoding the fucosyltransferase polypeptide is cloned into an expression vector which includes the dihydrofolate reductase (DHFR) gene. Integration of the plasmid and, therefore, the fucosyltransferase-encoding gene into the host cell chromosome is selected for by inclusion of 0.01-300 μ M methotrexate in the cell culture medium (as described in Ausubel et al., supra). This dominant selection can be accomplished in most cell types. Recombinant protein expression can be increased by DHFR-mediated amplification of the transfected gene. Methods for selecting cell lines bearing gene amplifications are described in Ausubel et al. (supra); such methods generally involve extended culture in medium containing gradually increasing levels of methotrexate. DHFR-containing expression vectors commonly used for this purpose include pCVSEII-DHFR and pAD26SV(A). (described in Ausubel et al., supra). Any of the host cells described above or, preferably, a DHFR-deficient CHO cell line (e.g., CHO DHFR⁻ cells, ATCC Accession No. CRL 9096) are among the host cells preferred for DHFR selection of a stably-transfected cell line or DHFR-mediated gene amplification.

Once the recombinant fucosyltransferase polypeptide is expressed, it is isolated, e.g., using affinity chromatography. In one example, an anti-fucosyltransferase antibody (e.g., produced as described below) may be attached to a column and used to isolate

- 26 -

- the polypeptide. Lysis and fractionation of fucosyltransferase-harboring cells prior to affinity chromatography may be performed by standard methods (see, e.g., Ausubel et al., supra). Once isolated, the recombinant protein can, if desired, be further purified, e.g., by high performance liquid chromatography (see, e.g., Fisher, *Laboratory Techniques In Biochemistry And Molecular Biology*, eds., Work and Burdon, Elsevier, 1980).
- 10 These general techniques of polypeptide expression and purification can also be used to produce and isolate useful fucosyltransferase fragments or analogs (described below).

15 Identification of Molecules Which Modulate Fucosyltransferase Expression

- Isolation of the fucosyltransferase gene also facilitates the identification of molecules which increase or decrease fucosyltransferase expression, and which are therefore useful as therapeutics, e.g., for treatment of inflammation. According to one approach, candidate molecules (e.g., peptide or non-peptide molecules found, e.g., in a cell extract, mammalian serum, or growth medium on which mammalian cells have been cultured, or oligonucleotides) are added at varying concentrations to the culture medium of cells which express fucosyltransferase mRNA (e.g., 32D c13). Fucosyltransferase expression is then measured by standard Northern blot analysis (Ausubel et al., supra) using fucosyltransferase cDNA as a hybridization probe.
- 30 The level of fucosyltransferase expression in the presence of the candidate molecule is compared to the level measured for the same cells in the same culture medium but in the absence of the candidate molecule. A molecule which promotes an increase or decrease in

- 27 -

fucosyltransferase expression is considered useful in the invention.

Anti-Fucosyltransferase Antibodies

- Fucosyltransferases described herein (or
- 5 immunogenic fragments or analogues) may be used to raise antibodies useful in the invention; such polypeptides may be produced by recombinant or peptide synthetic techniques (see, e.g., *Solid Phase Peptide Synthesis*, supra; Ausubel et al., supra). The peptides may be
- 10 coupled to a carrier protein, such as KLH as described in Ausubel et al, supra. The KLH-peptide is mixed with Freund's adjuvant and injected into guinea pigs, rats, or preferably rabbits. Antibodies may be purified by peptide antigen affinity chromatography.
- 15 Monoclonal antibodies may be prepared using the fucosyltransferase polypeptides described above and standard hybridoma technology (see, e.g., Kohler et al., *Nature* 256:495, 1975; Kohler et al., *Eur. J. Immunol.* 6:511, 1976; Kohler et al., *Eur. J. Immunol.* 6:292, 1976;
- 20 Hammerling et al., *In Monoclonal Antibodies and T Cell Hybridomas*, Elsevier, NY, 1981; Ausubel et al., supra).

- Once produced, polyclonal or monoclonal antibodies are tested for specific fucosyltransferase recognition by Western blot or immunoprecipitation analysis (by the
- 25 methods described in Ausubel et al., supra). Antibodies which specifically recognize fucosyltransferase are considered to be useful in the invention; such antibodies may be used, e.g., in an immunoassay to monitor the level of fucosyltransferase produced by a mammal.

30 Fucosylation and Production of Sialyl-Le^x Determinants

The invention features genes, enzymes, and methods for fucosylating virtually any protein bearing one or more glycan addition sites, e.g., an N-linked glycan

- 28 -

- addition site. By "N-linked" is meant bonded to the amide nitrogen of an asparagine residue of a protein. For example, it has been discovered that antibodies (as described in Seed et al., USSN 08/472,888, entitled "AGP-
- 5 Antibody Fusion Proteins and Related Molecules and Methods," filed June 7, 1995) bearing one or more genetically-engineered carbohydrate determinants mask the CH2 portion of the immunoglobulin molecule and thus inhibit complement fixation and F_c receptor binding.
- 10 Such antibodies are useful for disrupting undesirable interactions between cells or proteins, or, generally, for disrupting an interaction between any two molecules, one of which bears a determinant specifically recognized by an antibody. Because the carbohydrate moieties block
- 15 the immunoglobulin domain which triggers complement fixation and F_c receptor binding, such antibodies do not elicit the undesirable side effects (i.e., those resulting from complement fixation and F_c receptor binding) frequently associated with antibody-based
- 20 therapies. Preferably, the carbohydrate groups serve not only to inhibit undesirable complement fixation and F_c receptor binding, but also perform the function of competitively inhibiting a carbohydrate ligand-cell adhesion protein interaction. Where the carbohydrate
- 25 groups perform this function, the antibody generally does not serve any function arising from its specificity, but serves only as a carrier for the carbohydrate groups. There is described herein such a molecule, in which the carbohydrate side chain includes the sialyl- Le^x
- 30 determinant.

Sialyl- Le^x normally acts to facilitate interaction between cells which bear it (e.g., neutrophils) and cells which bear the protein, ELAM-1 or E-selectin (e.g., endothelial cells, e.g., those lining the blood vessel

35 walls). Disrupting this interaction has therapeutic

- 29 -

applications, for example, in minimizing inflammation, such as that which occurs following tissue injury, e.g., myocardial infarction, which is characteristic of diseases such as psoriasis or rheumatoid arthritis, or
5 for preventing or inhibiting septicemia or septic shock which is induced by a microbial- or host-mediated immune reaction.

According to one example, the gene encoding a protein bearing a sialyl-Le^x determinant, e.g., an IgG1
10 antibody or an α_1 -AGP-antibody fusion, is inserted into a vector designed to express the protein in a eukaryotic cell (see, e.g., those vectors described in Gillies et al., U.S. Patent No. 4,663,281, hereby incorporated by reference). The eukaryotic host cell is preferably a
15 mammalian cell (e.g., 32D c13, or human cell line 293, or a CHO, or lec11 cell), and the expression vector containing the sialyl-Le^x-encoding sequence is introduced into the host cell by transient or stable transfection using standard techniques. Such host cells are also
20 transfected (transiently or stably) with a vector capable of expressing an $\alpha(1,3)$ fucosyltransferase of the invention (i.e., an enzyme capable of attaching one or more sialyl-Le^x groups to the protein molecule at sialyl-Le^x consensus glycosylation sites (N-X-T/S)). The
25 $\alpha(1,3)$ fucosyltransferase gene described herein or a combination of the $\alpha(1,3)$ fucosyltransferase gene described herein and the Fuc-TIV gene may be expressed from a vector distinct from that encoding the protein containing sialyl-Le^x addition sites, or, if desired, the
30 genes may be carried on, and expressed from, a common vector. Mammalian cells are particularly useful hosts for the synthesis of sialyl-Le^x modified proteins because they provide all required precursors for sialyl-Le^x production.

- 30 -

Proteins (e.g., antibodies, AGP, or AGP-antibody fusions) which are fucosylated according to the methods of the invention have important therapeutic and diagnostic uses. Previous work has demonstrated that large amounts of antibody fusion proteins may be generated and secreted transiently from transfected mammalian cells (for example, COS cells). In general, to produce an antibody fusion protein, cDNA encoding a domain of interest is fused in-frame, for example, to human IgG domains (for example, constant domains) by standard techniques, and the fusion protein is expressed. The antibody portion of the molecule facilitates fusion protein purification and also prolongs the plasma half-life of otherwise short-lived polypeptides or polypeptide domains. Recombinant plasmids expressing α_1 -AGP-IgG1 fusion proteins (e.g., α_1 -AGP-Hinge-CH2-CH3 and α_1 -AGP-CH2-CH3) are disclosed in Seed et al., USSN 08/472,888, entitled "AGP-Antibody Fusion Proteins and Related Molecules and Methods," filed June 7, 1995.

Host cells expressing the $\alpha(1,3)$ fucosyltransferase of the invention or a combination of any $\alpha(1,3)$ fucosyltransferase of the invention and Fuc-TIV (e.g., SEQ ID NO: 4) along with a protein which is to be fucosylated, e.g., IgG1 or an AGP-antibody fusion, are grown by standard methods and the fucosylated protein purified by standard techniques (for example, for an antibody or antibody fusion protein, using a Protein A column).

Alternatively, any protein, e.g., IgG1 or an AGP-antibody fusion, bearing sialyl-Le^x addition sites may be fucosylated in vitro using any of the enzymes or any combination of enzymes described herein according to standard methods known in the art. Again, such in vitro fucosylated proteins can be purified using any standard technique of isolation and purification.

- 31 -

Fucosyltransferase Kits

Kits for carrying out any of the methods disclosed herein are also included in the invention. Such kits generally include a gene encoding the

- 5 $\alpha(1,3)$ fucosyltransferase of the invention (for example, a fucosyltransferase gene encoding a polypeptide including an amino acid sequence substantially identical to the amino acid sequence shown in Fig. 3; SEQ ID NO: 2). Such a kit may also include a gene encoding Fuc-TIV (for
- 10 example, a human Fuc-TIV polypeptide including an amino acid sequence substantially identical to the amino acid sequence shown in Fig. 6B; SEQ ID NO: 4) and/or a cell useful for expressing one or more fucosyltransferase genes. Alternatively, a kit according to the invention
- 15 may include a transformed cell harboring an $\alpha(1,3)$ fucosyltransferase gene described herein, optionally in combination with a Fuc-TIV-encoding gene. Preferably, such fucosyltransferases are expressed in the 32D c13 cell line or human cell line 293. For identifying
- 20 modulators of the fucosyltransferases described herein, a kit may include a fragment of an $\alpha(1,3)$ fucosyltransferase nucleic acid sequence useful for hybridization purposes, and may also include means for detecting and quantitating $\alpha(1,3)$ fucosyltransferase RNA hybridization.

- 25 Other kits according to the invention include substantially pure $\alpha(1,3)$ fucosyltransferase polypeptide (for example, a fucosyltransferase polypeptide including an amino acid sequence substantially identical to the amino acid sequence shown in Fig. 3; SEQ ID NO: 2). Such
- 30 a kit may also include substantially pure Fuc-TIV polypeptide (for example, a fucosyltransferase polypeptide including an amino acid sequence substantially identical to the amino acid sequence shown in Fig. 6B; SEQ ID NO: 4). Such fucosyltransferase kits
- 35 are useful for fucosylating a molecule in vitro.

- 32 -

Other Embodiments

Polypeptides according to the invention include the entire murine fucosyltransferase sequence (as shown in Fig. 3; SEQ ID NO: 2) as well as any analog or
5 fragment of the murine fucosyltransferase.

Polypeptides of the invention also include all mRNA processing variants (e.g., all products of alternative splicing or differential promoter utilization) as well as analogous fucosyltransferases
10 from other mammals, including humans.

Specific fucosyltransferase fragments or analogues of interest include full-length or partial (see below) proteins including an amino acid sequence which differs only by conservative amino acid substitutions, for
15 example, substitution of one amino acid for another of the same class (e.g., valine for glycine, arginine for lysine, etc.) or by one or more non-conservative amino acid substitutions, deletions, or insertions located at positions of the amino acid sequence which do not destroy
20 enzymatic activity (as assayed above or according to any other standard method). Analogs also include fucosyltransferase polypeptides which are modified for the purpose of increasing peptide stability; such analogs may contain, e.g., one or more desaturated peptide bonds
25 or D-amino acids in the peptide sequence or the peptide may be formulated as a cyclized peptide molecule.

Other embodiments are within the following claims.

- 33 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: The General Hospital Corporation
- (ii) TITLE OF INVENTION: FUCOSYLTRANSFERASE GENES AND USES THEREOF
- (iii) NUMBER OF SEQUENCES: 4
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Fish & Richardson P.C.
 - (B) STREET: 225 Franklin Street
 - (C) CITY: Boston
 - (D) STATE: MA
 - (E) COUNTRY: USA
 - (F) ZIP: 02110-2804
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/483,151
 - (B) FILING DATE: 07-JUN-1995
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Lech, Karen F.
 - (B) REGISTRATION NUMBER: 35,238
 - (C) REFERENCE/DOCKET NUMBER: 00786/278W01
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 617/542-5070
 - (B) TELEFAX: 617/542-8906
 - (C) TELEX: 200154

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1814 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GTAGCCAAGG TTCTCTCCA TCTACCAGA GCGTCTGGA GGGGAATCAA ACAAGCCTGG	60
ACCTGAGGCT GGGACTAGCT TTCTGTTC TGGAGTGGAT GCCAACCCCG TGCCACCCAG	120
CCTGCCTGTC CACGCCAGGG ACACACAGAC TCCTTCCCTT TCCAGACTGG AAAGCCCOCT	180
CCTGGGAGAG CAGGAAGGAA GCAACCTGCA ACTCTTCAG CCCTGGACCT TGGCTGAAC	240

- 34 -

CTACAGTTCA AGGGTGCCTC TGTGGAGAG GCTGCTGTGA TTTGAAAATC TTCITTCCTT	300
GGTGACAATT CCAGAAGGCT CCAGATGAAT TGTATTGGGT ACCACCCAC CAGGAGGCTG	360
CGGGCCTGGG GCGGCCTAGC TGGAGGAGCA ACATTTCATGG TAATTTGGTT TTTCTGGCTG	420
TGGGGATCAG CTCCTGGAAG TGCCCCGTGTG CCTCAGTCCA CACTCACCAT CCTTATCTGG	480
CACTGGCCTT TCACCAACCG GCGGCCAGAG CTACCTGGTG ACACCTGCAC TCGCTATGGC	540
ATGGCCAGCT GCCGTCTGAG TGCTAACCG AGCCTGCTAG CCAGTGCTGA TGCTGTGGTC	600
TTCCACCACC GTGAGCTGCA AACCCGGCAA TCTCTCTAC CCTCTGGACCA GAGGCCACAC	660
GGACAGCCTT GGGTCTGGGC CTCCTAGGAA TGGCCAGTA ATACCCATGG TCTCCATCGC	720
TTCCGGGGCA TCTTCAACTG GGTGCTGAGC TATCGGCGTG ATTGAGATAT CTTTGTACCC	780
TACGGTGGCT TGGAGCCTCT CTCTGGGCCC ACATCCCCAC TACCGGCCAA AAGCAGGATG	840
GCTGCCTGGG TGATCAGCAA TTTCCAGGAG CGGCAGCAGC GTGCAAAGCT GTACCGGCAG	900
CTGGCCCCCT ATCTGCAGGT GGATGTGTTT GGTGCGGCCA GCGGACGGCC CCTATGCGCT	960
AATTGTCTGC TGCCCCATTT GGCCCGGTAC CGCTTCTACC TGGCCTTTGA GAACTCACAG	1020
CATCGGGACT ACATCACTGA GAAGTTCTGG CGCAATGCCC TGGCGGCTGG TGCTGTACCC	1080
GTGGCGCTGG GACCTCTCTG GGCCACCTAC GAGGCTTTTG TGCCACCAGA TGCCCTTTGTA	1140
CACGTGGAAG ACTTCAGCTC TGCCCGTGAA CTGGCTGTCT TCCTCGTCAG CATGAATGAG	1200
AGTCGTTATC GTGGCTTCTT TGCTTGGCGA GACCGGCTCC GTGTGCGGCT CCTGGGTGAC	1260
TGGAGGGAGC GCTTCTGCAC CATCTGTGCC CGCTACCCCT ACTTGCCCGG CAGCCAGGTC	1320
TATGAAGACC TTGAAGACTG GTTCCAGGCT TGAACCTCTG CTGCTGGGAG AGGCTGGATG	1380
GGTGGGAGAC TGATGTTGAA ACCAAAGAGC TGGGCATCCA GCCTTTTGGT CACCATGGCA	1440
CTACCCCAAG GCTTTTCTCT TTCAGTGAGC AGGAATTCAG GATATAAGGA GAAAACCTGG	1500
CTGAGATGCC TGGTGGGCTT TAGAGTAGGG GCCCAGGATA AGAGACAATG AATTAATGAG	1560
GAGCATATGG GGAAGGTGGC TGAGGGTCCC TGACTTACCT TGACCCATGG CTGAAGGCTC	1620
CATGCCCATG GCTGGAGCTG GGACCCTACA CTCTATAGT CAAGGTGCTT AGCCTCAAGG	1680
TGTCAGATGC ACCCTCTAGT ACTCTGGGTG CAGACTGTAC ACTGGGCGCA GGGGTTGTG	1740
GAAGGACAGT GCAGATGATT CTGGGCTTTT GACACCACAG TTCCCCCAGG GAAAGAGGCA	1800
CTACTAATAA AAAC	1814

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 342 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

- 35 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Asn Cys Ile Gly Tyr His Pro Thr Arg Arg Leu Arg Ala Trp Gly
 1 5 10 15
 Gly Leu Ala Gly Gly Ala Thr Phe Met Val Ile Trp Phe Phe Trp Leu
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 Trp Gly Ser Ala Pro Gly Ser Ala Pro Val Pro Gln Ser Thr Leu Thr
 35 40 45
 Ile Leu Ile Trp His Trp Pro Phe Thr Asn Arg Pro Pro Glu Leu Pro
 50 55 60
 Gly Asp Thr Cys Thr Arg Tyr Gly Met Ala Ser Cys Arg Leu Ser Ala
 65 70 75 80
 Asn Arg Ser Leu Leu Ala Ser Ala Asp Ala Val Val Phe His His Arg
 85 90 95
 Glu Leu Gln Thr Arg Gln Ser Leu Leu Pro Leu Asp Gln Arg Pro His
 100 105 110
 Gly Gln Pro Trp Val Trp Ala Ser Met Glu Ser Pro Ser Asn Thr His
 115 120 125
 Gly Leu His Arg Phe Arg Gly Ile Phe Asn Trp Val Leu Ser Tyr Arg
 130 135 140
 Arg Asp Ser Asp Ile Phe Val Pro Tyr Gly Arg Leu Glu Pro Leu Ser
 145 150 155 160
 Gly Pro Thr Ser Pro Leu Pro Ala Lys Ser Arg Met Ala Ala Trp Val
 165 170 175
 Ile Ser Asn Phe Gln Glu Arg Gln Gln Arg Ala Lys Leu Tyr Arg Gln
 180 185 190
 Leu Ala Pro His Leu Gln Val Asp Val Phe Gly Arg Ala Ser Gly Arg
 195 200 205
 Pro Leu Cys Ala Asn Cys Leu Leu Pro Thr Leu Ala Arg Tyr Arg Phe
 210 215 220
 Tyr Leu Ala Phe Glu Asn Ser Gln His Arg Asp Tyr Ile Thr Glu Lys
 225 230 235 240
 Phe Trp Arg Asn Ala Leu Ala Ala Gly Ala Val Pro Val Ala Leu Gly
 245 250 255
 Pro Pro Arg Ala Thr Tyr Glu Ala Phe Val Pro Pro Asp Ala Phe Val
 260 265 270
 His Val Asp Asp Phe Ser Ser Ala Arg Glu Leu Ala Val Phe Leu Val
 275 280 285
 Ser Met Asn Glu Ser Arg Tyr Arg Gly Phe Phe Ala Trp Arg Asp Arg
 290 295 300
 Leu Arg Val Arg Leu Leu Gly Asp Trp Arg Glu Arg Phe Cys Thr Ile
 305 310 315 320

- 36 -

Cys Ala Arg Tyr Pro Tyr Leu Pro Arg Ser Gln Val Tyr Glu Asp Leu
 325 330 335

Glu Ser Trp Phe Gln Ala
 340

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 2134 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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GCGGGCTTGA	CGTGTACGGC	GCTGATCACC	TACGCTTGCT	GGGGGAGCT	CGCGCCGCTG	180
CCTGGGGGTC	GCCACCCCG	TGCGACCGG	TGGGCGTGCT	GCTGTGTGG	GAGCCCTTCG	240
GGGGCGCGAT	CAGCGCCCG	AGGCCGCCCC	CTGACTGCGG	GCTGCGCTTC	AACATCAGCG	300
GCTGCCGCCT	GCTCACCGAC	ACGCGCGTCC	TACGGAGAGG	CTCAGGCCGT	GCTTTTCCAC	360
CACCGCGACC	TGCTGAAGGG	GCCCCCGGAC	TGGCCCCCGC	CCTGGGGCAT	CCAGCGCGAC	420
ACTGCCGAGG	AGGTGGATCT	GCGGTGTTG	GACTACGAGG	AGCCAGCGGC	GGCGGCAGAA	480
GCCCTGGCGA	CCTCCAGCCC	CAGGCCCGCG	GCCAAGCGCT	GGCTTTGGAT	GAACTTCGAG	540
TGCGCCTCGC	ACTCCCCGGG	GCTGCGAAGC	CTGGCAAGTA	ACCTCTTCAA	CTGGACGCTC	600
TCCTACGGGG	CGGACTCGGA	CGTCTTTGTG	CCTTATGGCT	ACCTCTACCC	CAGAAGCCAC	660
CCGGGCGACC	CGCCCTCAGG	CCTGGCCCCG	CCACTGTCCA	GGAAACAGGG	GCTGGTGCCA	720
TGGGTGGTGA	GCCACTGGGA	CGAGCGCCAG	GCCCGGGTCC	GCTACTACCA	CCAAGTGCAG	780
CAACATGTGA	CGGTGGACGT	GTTCGGCCGG	GGCGGGCCGG	GGCAGCCGGT	GCCCGAATT	840
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CTGGATTATA	TCACCGAGAA	GCTCTGGCGC	AACGCGTTGC	TGCTGGGGC	GGTGCCGGTG	960
GTGCTGGGCC	CAGACCGTGC	CAACTACGAG	CGCTTTGTGC	CCCGCGCGCG	CTTCATCCAC	1020
GTGGACGACT	TCCCAAGTGC	CTCCTCCCTG	GCCTCGTACC	TGCTTTTCCT	CGACGCGAAC	1080
CCCGGGTCT	ATCGCCGCTA	CTTCCACTGG	CGCCGAGCT	ACGCTGTCCA	CATCACCTCC	1140
TTCTGGGACG	AGCCTTGGTG	CGGGGTGTGC	CAGGCTGTAC	AGAGGGCTGG	GACCGGCCCCA	1200
AGAGCATACG	GAACTTGGCC	AGCTGGTTCG	AGCGGTGAAG	CGCGCTCCC	CTGGAAGCGA	1260

- 37 -

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CCCAGGGGAG GCCAAGTTGT CAGCTTTTTC ATCCTCTACT GTGCATCTCC TTGACTGCCC 1320
GCATCATGGG AGTAAGTTCT TCAACACCCC ATTTTGTCTC TATGGGAAAA AACGATTTA 1380
CCATTAATAT TTACTCAGCA CAGAGATGGG GGCCCGGTTT CCATATTTT TGCACAGCTA 1440
GCAATTGGGC TCCCTTTGCT GCTGATGGGC ATCATTGTTT AGGGGTGAAG GAGGGGGTTC 1500
TTCTTCACCT TGTAAACCACT GCAGAAATGA AATAGCTTAG CGCAAGAAGC CGTTGAGGCG 1560
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GCTGACTTCT TTCACAAGTA CTATCTGTTT CCCTGTCTCT TGAATGGAAG CAAAGTGCTG 1860
GATTGTCCTT GGAGGAAACT TAAGATGAAT ACATGCGTGT ACCTCACTTT ACATAAGAAA 1920
TGTATTCCTG AAAAGCTGCA TTAAATCAA GTCCCAAATT CATTGACTTA GGGGAGTTCA 1980
GTATTTAATG AAACCCATG GAGAATTAT CCCTTTACAA TGTGAATAGT CATCTCCTAA 2040
TTTGTTCITT CTGTCITTAT GTTTTCTAT AACCTGCATT TTTAAATCA TATTAATAAT 2100
ACAGATGTGA AATAAAAAA AAAAAAAAAA AAAA 2134

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(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 405 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: not relevant
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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 20           25           30
Ala Ala Gly Leu Thr Cys Thr Ala Leu Ile Thr Tyr Ala Cys Trp Gly
 35           40           45
Gln Leu Pro Pro Leu Pro Trp Ala Ser Pro Thr Pro Ser Arg Pro Val
 50           55           60
Gly Val Leu Leu Trp Trp Glu Pro Phe Gly Gly Ala Ile Ser Ala Pro
 65           70           75           80
Arg Pro Pro Pro Asp Cys Arg Leu Arg Phe Asn Ile Ser Gly Cys Arg
 85           90           95

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- 38 -

Leu Leu Thr Asp Arg Ala Ser Tyr Gly Glu Ala Gln Ala Val Leu Phe
 100 105 110
 His His Arg Asp Leu Val Lys Gly Pro Pro Asp Trp Pro Pro Pro Trp
 115 120 125
 Gly Ile Gln Ala His Thr Ala Glu Glu Val Asp Leu Arg Val Leu Asp
 130 135 140
 Tyr Glu Glu Ala Ala Ala Ala Glu Ala Leu Ala Thr Ser Ser Pro
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 Arg Pro Arg Ala Lys Arg Trp Val Trp Met Asn Phe Glu Ser Pro Ser
 165 170 175
 His Ser Pro Gly Leu Arg Ser Leu Ala Ser Asn Leu Phe Asn Trp Thr
 180 185 190
 Leu Ser Tyr Arg Ala Asp Ser Asp Val Phe Val Pro Tyr Gly Tyr Leu
 195 200 205
 Tyr Pro Arg Ser His Pro Gly Asp Pro Pro Ser Gly Leu Ala Pro Pro
 210 215 220
 Leu Ser Arg Lys Gln Gly Leu Val Ala Trp Val Val Ser His Trp Asp
 225 230 235 240
 Glu Arg Gln Ala Arg Val Arg Tyr Tyr His Gln Leu Ser Gln His Val
 245 250 255
 Thr Val Asp Val Phe Gly Arg Gly Gly Pro Gly Gln Pro Val Pro Glu
 260 265 270
 Ile Gly Leu Leu His Thr Val Ala Arg Tyr Lys Phe Tyr Leu Ala Phe
 275 280 285
 Glu Asn Ser Gln His Leu Asp Tyr Ile Thr Glu Lys Leu Trp Arg Asn
 290 295 300
 Ala Leu Leu Ala Gly Ala Val Pro Val Val Leu Gly Pro Asp Arg Ala
 305 310 315 320
 Asn Tyr Glu Arg Phe Val Pro Arg Gly Ala Phe Ile His Val Asp Asp
 325 330 335
 Phe Pro Ser Ala Ser Ser Leu Ala Ser Tyr Leu Leu Phe Leu Asp Arg
 340 345 350
 Asn Pro Ala Val Val Arg Arg Tyr Phe His Trp Arg Arg Ser Tyr Ala
 355 360 365
 Val His Ile Thr Ser Phe Trp Asp Glu Pro Trp Cys Arg Val Cys Gln
 370 375 380
 Ala Val Gln Arg Ala Gly Asp Arg Pro Lys Ser Ile Arg Asn Leu Ala
 385 390 395 400
 Ser Trp Phe Glu Arg
 405

- 39 -

Claims

1. Substantially pure $\alpha(1,3)$ fucosyltransferase comprising an amino acid sequence substantially identical to the amino acid sequence shown in Fig. 3 (SEQ ID NO: 5 2).
2. A fragment or analog of $\alpha(1,3)$ fucosyltransferase polypeptide comprising an amino acid sequence substantially identical to the amino acid sequence shown in Fig. 3 (SEQ ID NO: 2).
- 10 3. The polypeptide of claim 1, wherein said polypeptide is derived from a mammal.
4. The polypeptide of claim 3, wherein said cell is of murine or human origin.
5. The polypeptide of claim 4, wherein said 15 murine cell is 32D cl3.
6. Substantially pure DNA having a sequence substantially identical to the nucleotide sequence shown in Fig. 3 (SEQ ID NO: 1).
7. The DNA of claim 6, wherein said DNA is cDNA.
- 20 8. A vector comprising the DNA of claim 6.
9. A cell containing the vector of claim 8, said vector being capable of directing expression of the protein encoded by said DNA in a vector-containing cell.
10. A cell which contains the DNA of claim 6.

- 40 -

11. The cell of claim 10, said cell being a eukaryotic cell.

12. The cell of claim 11, wherein said cell is 32D c13 or human cell line 293.

5 13. A method of fucosylating a polypeptide in vivo comprising:

- (a) providing a cell of claim 10; and
 - (b) culturing said transformed cell under conditions for expressing said DNA, wherein said
- 10 expression results in the fucosylation of said polypeptide.

14. The method of claim 13, wherein said cell is a human or murine cell.

15 15. The method of claim 14, wherein said cell is human cell line 293 or a 32D c13 cell.

16. The method of claim 13, wherein said cell contains a second fucosyltransferase gene.

17. The method of claim 16, wherein said second fucosyltransferase gene is substantially identical to the

20 nucleotide sequence shown in Fig. 6A (SEQ ID NO: 3) which encodes a polypeptide comprising an amino acid sequence substantially identical to the amino acid sequence shown in Fig. 6B (SEQ ID NO: 4).

25 18. The method of claim 13, wherein said polypeptide is an antibody or an AGP-antibody fusion protein.

- 41 -

19. A recombinant polypeptide fucosylated using a cell expressing the DNA of claim 6.

20. The polypeptide of claim 19, wherein said fucosylated polypeptide is an antibody or an AGP-antibody fusion protein.

21. The polypeptide of claim 19, wherein said polypeptide is further fucosylated using a second fucosyltransferase.

22. The polypeptide of claim 21, wherein said second fucosyltransferase is substantially identical to a polypeptide comprising an amino acid sequence shown in Fig. 6B (SEQ ID NO: 4).

23. A polypeptide fucosylated in vitro using a fucosyltransferase having an amino acid sequence substantially identical to the sequence shown in Fig. 3 (SEQ ID NO: 2).

~~24. The polypeptide of claim 23, wherein said~~
polypeptide is further fucosylated using a second fucosyltransferase.

25. The polypeptide of claim 24, wherein said second fucosyltransferase comprises an amino acid sequence substantially identical to the sequence shown in Fig. 6B (SEQ ID NO: 4).

26. The polypeptide of claim 23, wherein said fucosylated protein is an AGP-antibody fusion protein.

- 42 -

27. Substantially pure polypeptide of claim 19 or claim 23, wherein said polypeptide is capable of protecting a mammal against an adverse immune reaction.

28. The protein of claim 27, wherein said adverse
5 immune reaction is septic shock or septicemia.

29. A cell containing at least two recombinant fucosyltransferases, one of said fucosyltransferases being substantially identical to the amino acid sequences shown in Fig 3. (SEQ ID NO: 2) and another of said
10 fucosyltransferases being substantially identical to the amino acid sequence shown in Fig. 6B (SEQ ID NO: 4).

30. A method of fucosylating a polypeptide in vitro comprising:

- (a) providing an $\alpha(1,3)$ fucosyltransferase of
15 claim 1; and
(b) contacting said polypeptide with said fucosyltransferase under conditions sufficient for fucosylating said polypeptide.

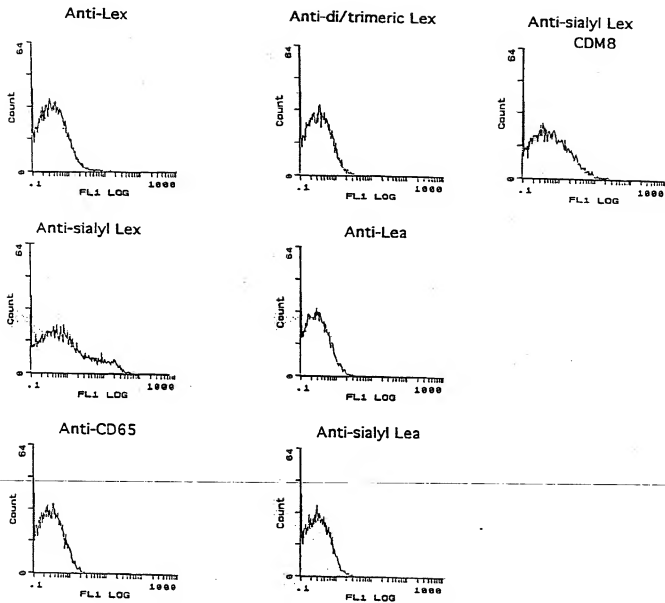


Fig. 1

2/10



Fig. 2A

3/10

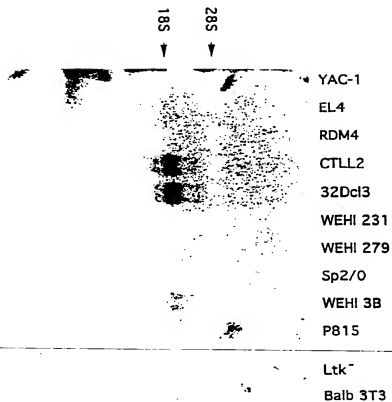


Fig. 2B

4/10

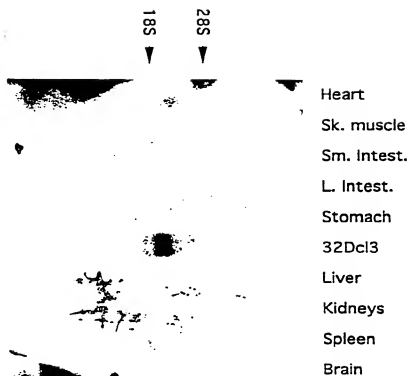


Fig. 2C

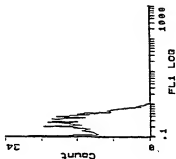
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 M N C I G Y H P T R R L
 361 cgggctggggcgccctagctggaggagcaacattcatggtaatttgggttttctggctg
 R A W G G L A G G A T F M V I W F F W L
 421 tggggatcagctccttgaagtgcctctgtgcctcagtcacacatccacatccttatctgg
 W G S A P G S A P V P Q S T L T I L I W
 481 catgggcctttcaccaaccggcgccagagctacctgggtgacacctgcaactcgtatggc
 H W P F T N R P P E L P G D T C T R Y G
 541 atggccagctgcctctctgagtgctaaacggagcctgctagccagtgctgctgtgtggctc
 M A S C R L S A N R S L L A S A D A V V
 601 ttccaccaccgtgagctgcaaaccccggaatctctcctaccctggaccagaggccacac
 F H H R E L Q T R Q S L L P L D Q R P H
 661 ggcagaccccttgggtctgggcctccatggaatcgccagtaataccatggtctccatcgc
 G Q P W V W A S M E S P S N T H G L H R
 721 tccggggcatcttcaactgggtgctgagctatcggcgtgattcagatatcttctgtaccc
 F R G I F N W V L S Y R R D S D I F V P
 781 taeggtcgttggagcctctctctggggccacatccccactaccggccaaaaggcaggatg
 Y G R L E P L S G P T S P L P A K S R M
 841 gctgctgggtgatcagcaatttccaggagcggcagcagcgtgcaaaagctgtaccggcag
 A A W V I S N F Q E R Q Q R A K L Y R Q
 901 ctggccctccatctgcaggtggatgtgttcggtcgcccgagcggacggccccctatgcgct
 L A P H L Q V D V F G R A S G R P L C A
 961 aattgtctgctccccactttggcccggtaccgcttctacctggcctttgagaactcacag
 N C L L P T L A R Y R F Y L A F E N S Q
 1021 catcggtactacatcactgagaagtcttggcgcaatgccctggcggtgctgctgtaccc
 H R D Y I T E K F W R N A L A A G A V P
 1081 gtggcctgggacctcctcgggccacctacgaggtctttgtgccaccagatgcctttgta
 V A L G P P R A T Y E A F V P P D A F V
 1141 cactggagcagcttcagctctgcccgtaactggctgtcttctcgtcagcatgaatgag
 H V D D F S S A R E L A V F L V S M N E
 1201 agtcgttatcgtggtcttcttgccttggcgagaccggctccgtgtgcggctccttgggtgac
 S R Y R G F F A W R D R L R V R L L G D
 1261 tggaggggagcgtcttctgcaccatctgtgcccgcctacccttacttgccccgcagcaggct
 W R E R F C T I C A R Y P Y L P R S Q V
 1321 tatgaagaccttgaaagctgggtccaggcttgaaactcctgctgctgggagaggtcggatg
 Y E D L E S W F Q A *
 1381 ggtgggagactgatgttgaaaccaaagagctgggcatccaggcttcttgggtcaccatggca
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Fig. 3

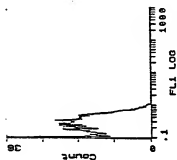
(SEQ ID NOS: 1, 2)

Fig. 5

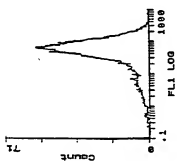
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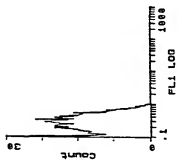
Anti-CD65



Anti-sialyl Lex

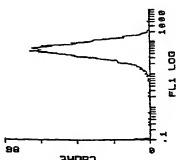
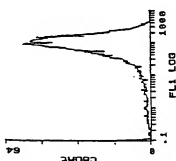
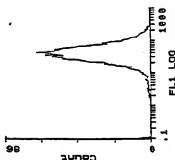
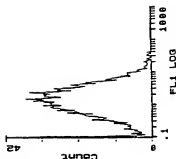


Anti-Lex

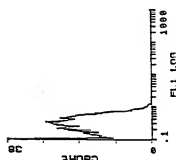


32D C13

32D F13IV (myeloid)



Anti-mouse IgG Ctrl



Anti-mouse IgM Ctrl1

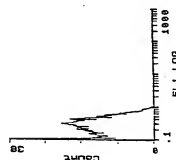


Fig. 5 A

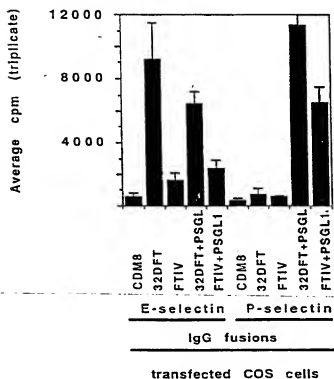


Fig. 5 B

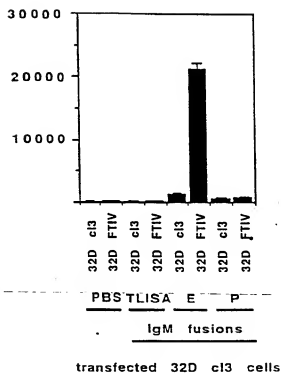


Fig. 6^{8/10} A

SEQ ID NO: 3

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51 GGC GGCGGGG GGGCGGCGCG GGTGGCGCCG AGGCCGGGG CTGCCATGGA
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151 TACGCTTGCT GGGGGCAGCT GCCGCCGCTG CTTGGGCGTC GCCAACCCCG
201 TCGCGACCGG TGGGCGTGCT GCTGTGGTGG GAGCCCTTCG GGGGCGCGAT
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301 GCTGCCGCCT GCTCACCGAC ACGCGCGTCC TACGGAGAGG CTCAGGCCGT
351 GCTTTTCCAC CACCGCGACC TCGTGAAGGG GCCCCCGAC TGGCCCCCGC
401 CCTGGGGCAT CCAGGCGCAC ACTGCCGAGG AGGTGGATCT GCGCGTGTG
451 GACTACGAGG AGGCAGCGGC GGCGGCAGAA GCCCTGGCGA CCTCCAGCCC
501 CAGGCCCCGG GCCAAGCGCT GGGTTTGGAT GAACTTCGAG TCGCCCTCGC
551 ACTCCCCGGG GCTGCGAAGC CTGGCAAGTA ACCTCTTCAA CTGAGCGCTC
601 TCCTACCGGG CGGACTCGGA CGTCTTTGTG CCTATGGCT ACCTCTACCC
651 CAGAAGCCAC CCCGGCGACC CGCCCTCAGG CTTGGCCCCG CCACTGTCCA
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751 GCCCGGTTC GCTACTACCA CCAACTGAGC CAACATGTGA CCGTGGACGT
801 GTTCGGCCGG GCGGGGCCGG GGCAGCCGGT GCCCGAAATT GGGCTCCTGC
851 ACACAGTGGC CCGCTACAAG TTCTACCTGG CTTTCGAGAA CTCGCAGCAC
901 CTGGATTATA TCACCGAGAA GCTCTGGCGC AACGCGTTGC TCGCTGGGGC
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1001 CCCGCGGCGC CTTTCATCCAC GTGGACGACT TCCCAAGTGC CTCCTCCCTG
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1101 CTTCCACTGG CGCCGGAGCT ACGCTGTCCA CATCACCTCC TTCTGGGAGC
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1251 CTGGAAGCGA CCCAGGGGAG GCCAAGTTGT CAGCTTTTTC ATCCTCTACT

9/10

1301 GTGCATCTCC TTGALTGCCC GCATCATGGG AGTAAGTTCT TCAAACACCC
1351 ATTTTGTCTC TATGGGAAAA AAACGATTTA CCAATTAATA TTACTCAGCA
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1451 TCCCTTTGCT GCTGATGGG ATCATTGTTT AGGGGTGAAG GAGGGGGTTC
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2101 ACAGATGTGA AAATAAAAAA AAAAAAAAAA AAAA

Fig. 6 A

SEQ ID NO: 4

1 MGAPWGSPTA AAGGRRGWRR GRGLPWTVCV LAAAGLTCTA LITYACWGQL
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201 VFVPYGYLYP RSHPGDPPSG LAPPLSRKQG LVAWVVSHPD ERQARVRYH
251 QLSQHVTVDV FGRGGPGQPV PEIGLLHTVA RYKFYLAFEN SQHLDYITEK
301 LWRNALLAGA VPVVLGPDRA NYERFVPRGA FIHVDDFPSA SSLASYLLFL
351 DRNPAVYRRY FHWRRSYAVH ITSFWDEPWC RVCQAVQRAG DRPKSIRNLA
401 SWFER*

Fig. 6 B

International application No.
PCT/US96/06427

IPC(6) : C12N 9/10, 9/64; C12P 21/06
US CL : 435/226, 193, 69.1,

According to International Patent Classification (IPC) or to both national classification and IPC

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/226, 193, 69.1.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN (medline, caplus, wpids). Search terms: Fucosyltransferase; Fucosyltransferase and amino acid sequence, .. and DNA.. and murine ... and Human.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Journal Biol. Chem., Volume 269, Number 20, issued 20 May, 1994, Sasaki et al., "Expression Cloning of a Novel alpha1,3-Fucosyltransferase that is Involved in the Biosynthesis of Sialyl Lewis X Carbohydrate", pages 14730-14737. 81.3% query match in protein database search - MasPar. Nucleotide and deduced amino acid sequence of a novel 1,3-fucosyltransferase, see Fig 2., page 14734.	1-5 and 30

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

- | | | | |
|--|---|-----|--|
| Special categories of cited documents: | | "T" | later documents published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be of particular relevance | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" | earlier document published on or after the international filing date | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
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| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date | | |

Date of the actual completion of the international search

19 JULY 1996

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
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Date of mailing of the international search report

19 AUG 1996

Authorized officer

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Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/06427

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Journal Biol. Chem., Volume 269, Number 24, Issued 17 June, 1994, Natsuka et al., "Molecular Cloning of a cDNA Encoding a Novel Human Leukocyte alpha 1,3-Fucosyltransferase Capable of Synthesizing the Sialyl Lewis x Determinant", pages 16789-16794. Novel DNA and Amino acid sequences of human leukocyte alpha 1,3-fucosyltransferase, see Fig.1., page 16791. Protein sequence query match - 79.7%.	1-5 and 30
X	Journal Biol. Chem. Volume 267, Number 34, Issued 05 December, 1992, Weston et al., " Molecular cloning of a Forth Member of a Human alpha 1, 3- fucosyltransferase gene", pages 24575-24584, Fig.1. DNA and protein sequences are disclosed. Fig. 2, shows four cloned human alpha 1,3-fucosyltransferase.	1-5 and 30
Y	Journal Biol. Chem. Volume 269, Number 2, Issued 14 January, 1994, Goetz et al., "Differential Expression of an E-selectin Ligand alpha 1,3- Fucosyltransferase Gene (ELET)", pages 1033-1040.	30
X	WO, A, 94/23021 (KYOW HUKKO KOYO KK) 13 October 1994, page 1, abstract.	1-5 and 30

Form PCT/ISA/210 (continuation of second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/06427**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(e) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-5 and 30

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1))(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/06427

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

- Group I, claims 1-5 and 30, drawn to a fucosyltransferase "substantially identical" to SEQ ID NO:2.
- Group II, claims 6-18 and 29, drawn to DNA "substantially identical" to SEQ ID NO:1, vectors and host cells comprising said DNA and a method of using said DNA.
- Group III, claims 19, 20, 23, and 26-28, drawn to a fucosylated protein.
- Group IV, claims 21, 22, 24, and 25, drawn to a doubly fucosylated protein.

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The claims of Group I share a technical feature of a fucosyltransferase "substantially identical" to the amino acid sequence of SEQ ID NO:2 (i.e., sharing at least 50% sequence identity with SEQ ID NO:2, see page 5, lines 15-30 of the disclosure). The claims of Group II share a technical feature of DNA "substantially identical" to SEQ ID NO:1. The claims of Group III share a technical feature of fucosylation and the claims of Group IV share a technical feature of double fucosylation.

Fucosyltransferases sharing at least 50% sequence identity with, and therefore "substantially identical" to, SEQ ID NO:2 were known in the prior art. See Sasaki et al. (Journal of Biological Chemistry, Vol. 269, No. 20, issued 20 May 1994, pages 14730-14737) and Natsuka et al. (Journal of Biological Chemistry, Vol. 269, No. 24, issued 17 June 1994, pages 16789-16794). In addition, fucosylated and multiply fucosylated proteins were known in the art (see, e.g., Sasaki et al., Figure 5 and Table II, and Natsuka et al., Figure 3 and Table II). The various Groups of inventions thus do not share a technical relationship involving one or more of the same or corresponding special technical features, i.e., those technical features that define a contribution which each invention, considered as a whole, makes over the prior art. They therefore do not fulfill the requirements of unity of invention and a holding of lack of unity for examination purposes is proper.